



Research article

Laboratory and clinico-demographic profile of patients investigated for tuberculosis in the National Referral Hospital of Bhutan

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ARTICLE INFO

Keywords:

Public health
Respiratory system
Pathology
Laboratory medicine
Clinical research
Infectious disease
Diagnosis
Bhutan
Sputum
Tuberculosis, pulmonary, extra-pulmonary, microscopy

ABSTRACT

Tuberculosis (TB), caused by the bacterium *Mycobacterium tuberculosis*, is an important public health problem in Bhutan. Microscopy is the primary method of diagnosis of TB in developing countries including Bhutan. Performance of microscopy in the Jigme Dorji Wangchuck National Referral Hospital (JDWRH), has never been assessed. A retrospective review of laboratory records for three years (2014–2016) was performed to determine the laboratory profile of patients investigated for different types of TB at the JDWRH.

A total of 10,821 sputum and 3,495 non-sputum samples were examined for pulmonary TB (PTB) and extrapulmonary TB (EPTB) respectively. The commonest EPTB samples were Fine Needle Aspiration Cytology (FNAC), urine and sterile fluids. About 6% (127/2163), 5% (130/2390) and 5% (289/5310) were positive for PTB in 2014, 2015 and 2016 respectively and EPTB positivity was about 7% in all years. During follow-up a significant number of patients remained sputum positive. Sputum sample satisfactory rate (quality) varied between 51% to 79% in the primary samples. Sample completeness (number) ranged between 62.3% to 94.6% but dropped sequentially in the follow-up cases. Sample completeness of urine samples for EPTB ranged between 75–90%. EPTB positivity rate was highest in FNAC, followed by urine, pleural fluid and ascitic fluid samples.

Higher number of patients were investigated for TB in subsequent years from 2014 to 2016. TB positivity rates for PTB and EPTB remained consistent over three years at about 5–6% and 7% respectively. There was a significant variation in sputum sample adequateness (by quality and number). Sputum conversion in the follow-up cases was lower than other countries. Educating the patients on the importance of providing adequate samples can improve TB diagnosis, enhance early treatment, reduce transmission and contribute significantly towards TB elimination.

1. Introduction

In the year 2014, tuberculosis (TB) killed 1.5 million people comprising of 890,000 men, 480,000 women and 140,000 children. In the same year, 9.6 million people were estimated to have fallen ill with TB worldwide [1]. The HIV epidemic had a huge impact on the epidemiology of TB, driving up incidence rates dramatically especially in sub-Saharan Africa. TB is one of the public health problems in Bhutan. The National TB Control Program (NTCP) was established in 1986 and Bhutan adopted the World Health Organization (WHO) recommended Directly Observed Treatment Short-course (DOTS) strategy in 1997. According to the Global TB Control Report 2012, the TB incidence in

Bhutan was estimated at 191 per 100,000 population [2]. The tuberculosis prevalence rate for the country has remained almost stable at 15, 16, 15, 15 and 13 per 10,000 population for the years 2011, 2012, 2013, 2014 and 2015 respectively [3]. The case notification rate of all forms of TB in 2011 was 174/100 000, with a treatment success rate of 90% [1]. Despite multiple strategies adopted by the NTCP, tuberculosis of all forms remain a public health challenge in the country. This challenge has recently heightened due to the emergence of multidrug-resistant TB (MDR-TB) and increasing people living with Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS). A surveillance by the Royal Centre for Disease Control (RCDC) of Bhutan reported a drug resistance to any of the primary TB drugs at 25%, any resistance to

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rifampicin at 10.11% and to isoniazid at 21.58% amongst newly diagnosed TB patients. Similarly, among previously treated cases, about 55% showed resistance to at least one primary drug, 44.2% resistance to rifampicin and 48.84% to isoniazid [4]. Most MDR-TB patients in Bhutan were males (63%) [5]. Extrapulmonary TB (EPTB) contributed up to 30%–40% of the total TB cases in Bhutan with TB lymph node and pleural effusion being the commonest forms of EPTB [6]. Another study documented that 14% of TB cases in Bhutan were children aged <15 years [7].

Sputum microscopy is the most important and widely available test for the diagnosis of pulmonary tuberculosis in low and middle-income countries, where 95% of tuberculosis cases and 98% of deaths occur [8]. Most laboratories in developing countries including Bhutan still use smears of un-concentrated sputum (direct smears) with Ziehl-Neelsen (ZN) staining for Acid Fast Bacilli (AFB). The ZN stain and microscopic examination method is also used for the diagnosis of non-pulmonary tuberculosis in most cases although not as sensitive. Microscopy is fast, simple, inexpensive, widely applicable, and highly specific for *M. tuberculosis* in TB endemic countries. It is believed that concentrating the sputum sample may increase the sensitivity of sputum microscopy but there are not many studies to support this. In a study to compare the sensitivity of unconcentrated and concentrated sputum, there was only a percent increase in sensitivity (51% vs 52% for unconcentrated and concentrated samples) when used alone. However, when used together, the overall sensitivity increased to 64% [9]. Sputum microscopy is highly dependent on the expertise of the microscopist and the commonly accepted sensitivity is only about 53%. Nevertheless, positive microscopy findings in TB endemic areas have a specificity of as high as 99% [10]. Urine microscopy for AFB has questionable sensitivity and specificity due to contamination from environmental saprophytic mycobacteria and lack recent studies with questionable utility.

Current WHO and international guidelines recommend the examination of three sputum samples (spot, morning, spot) [11] and this practice is followed by the Bhutan NTCP for the diagnosis of PTB [12]. Patients suspected of genitourinary TB are also investigated with three early morning urine samples for AFB. PTB patients on treatment are reviewed at two, four and six (on treatment completion) months with a repeat sputum microscopy [12]. In addition to the number of samples, the quality of sputum sample is routinely assessed during sample reception. To date, studies and data on the proportions of sample completeness and quality are scarce and has not been studied in Bhutanese laboratories as well. This study aimed to describe the demography of patients investigated for tuberculosis, ascertain the adequacy of sputum samples by number and quality, the proportion of positive and negative microscopy results and compare with international accepted microscopy positivity rates and understand the time taken for sputum negativity after initiating treatment. The study is timely and relevant since the Ministry of Health, Royal Government of Bhutan is aiming to eliminate TB in the country by the year 2030.

2. Materials and methods

2.1. Study design

This descriptive retrospective study reviewed secondary data from the TB laboratory, Microbiology unit of the Department of Laboratory Medicine, Jigme Dorji Wangchuck National Referral Hospital in Thimphu, Bhutan. The data were extracted from the TB register from 2014 to 2016, by the researchers who are employees of the hospital laboratory. Socio-demographic characteristics including age, sex, patient location (outpatient or in-patient), type and number of samples and microscopy examination findings were extracted from the hospital data repository.

In routine practice, patients requiring investigation for TB are directed by clinicians to submit sputum samples to the laboratory. The laboratory unit supplies sample containers, give instructions for sample collection and subsequently receive the samples for ZN staining and

examination. The first samples are taken as primary and subsequent samples (in follow up patients) were taken as second or third samples as indicated. For completeness of sputum samples, the WHO recommendation of three sputum samples was considered and defined as “complete” (if three samples submitted) or “incomplete” (if only one or two samples submitted). Quality of sputum samples were graded as “satisfactory” (mucoid/blood-stained samples) or “unsatisfactory” (salivary/watery sample). No entries in the laboratory register were excluded for missing data in a specific variable but excluded from the statistical calculation of that particular variable. EPTB samples like urine are collected by the laboratory but other samples like FNAC, sterile fluids and CSF are collected by relevant clinical units and sent to the laboratory for examination. Quality and reliability of microscopic findings and thereby the data of this study, as extracted from the registers were considered of acceptable quality since the laboratory is a national referral hospital with experienced laboratory staff and also routinely participate in external quality assessment from the National Tuberculosis Reference Laboratory, the Royal Center for Disease Control of Bhutan (<http://www.rcdc.gov.bt/web/laboratory-service/>).

2.2. Data analysis

Data were entered into an excel sheet and analyzed using STATA software version 15 (Stata Corporation, College Station, TX, USA). A descriptive analysis on demographic characteristics, proportions of different samples for each year, sputum positivity rate, sputum conversion rates and adequateness of samples were carried out.

2.3. Ethical clearance

Ethical approval was accorded by the Research Ethics Board of Health, Ministry of Health, Bhutan (REBH/Approval/2016/059). Informed consent was not required since this was a retrospective review of laboratory registers involving no patient contact. Data were anonymized to avoid identification of the patients.

3. Results

A total of 10,821 pulmonary (sputum) and 3,495 extrapulmonary samples were collected during the three-year study period (2014–16). The highest number of samples were collected in 2016 for both PTB and EPTB investigations. PTB samples approximately made up three-quarter of the total samples for all the three years. The commonest age group of patients was 26–35 years for both PTB and EPTB in all three years contributing to around 20% of the total samples. Children under 12 years contributed to less than 10% of PTB samples but ranged between 7 to 15% for the EPTB samples (Table 1).

A variety of EPTB samples were collected from different parts of the body. The highest EPTB sample was collected in 2016 with 1,513 followed by 1,186 in 2015. In 2014, urine was the commonest EPTB sample comprising 36.3% (289) followed by Fine Needle Aspiration Cytology (FNAC) 30.7% (244). However, in 2015 and 2016, FNAC was the commonest EPTB sample with 50.1% (594) and 52.6% (796). Overall, the most common EPTB sample examined was FNAC followed by urine and sterile fluids. Extra-pulmonary samples positivity rate for TB was highest in FNAC, followed by urine, pleural fluid and ascitic fluid but the overall positivity rate remained consistent over all the years at about 7% (Table 2).

More than 70% of the samples were collected from the Outpatient departments (OPD) for both PTB and EPTB. Almost an equal number of males and females were investigated for PTB and EPTB in all the three years (Figure 1).

Of the total patients investigated for PTB in three years, 5.9% (127/2163), 5.4 % (130/2390) and 5.4% (289/5310) were positive for tuberculosis in the year 2014, 2015 and 2016 respectively. Sputum conversion rate during the first follow up (2 months) in 2014, 2015 and

Table 1. Type of samples examined stratified by age group and patient location (2014–16).

Year	2014		2015		2016	
	PTB (%)	EPTB (%)	PTB (%)	EPTB (%)	PTB (%)	EPTB (%)
A. By age group (Years)						
0-12	48 (2.2)	51 (6.5)	76 (3.2)	129 (11.2)	165 (3.1)	216 (15.1)
13-18	192 (9.0)	65 (8.2)	179 (7.5)	88 (7.6)	383 (7.2)	132 (9.2)
19-25	435 (20.3)	128 (16.2)	460 (19.3)	189 (16.3)	1,031 (19.5)	239 (16.7)
26-35	437 (20.4)	182 (23.1)	461 (19.3)	247 (21.4)	1,065 (20.1)	288 (20.2)
36-45	253 (11.8)	119 (15.1)	278 (11.6)	192 (16.6)	596 (11.3)	177 (12.4)
46-55	243 (11.3)	86 (10.9)	264 (11.1)	113 (9.8)	537 (10.1)	162 (11.3)
56-65	239 (11.2)	73 (9.3)	254 (10.6)	104 (9.0)	583 (11.0)	92 (6.4)
>65	295 (13.8)	85 (10.8)	417 (17.5)	95 (8.2)	940 (17.7)	123 (8.6)
Total	2142 (100.0)	789 (100.0)	2389 (100.0)	1157 (100.0)	5300 (100.0)	1429 (100.0)
B. By patient location						
Inpatients	418 (19.3)	243 (30.5)	579 (24.2)	311 (26.2)	1,326 (25.0)	353 (23.3)
Outpatients	1,745 (80.7)	553 (69.5)	1,811 (75.8)	785 (73.8)	3,984 (75.0)	1,160 (76.7)
Total	2163 (100.0)	796 (100.0)	2390 (100.0)	1096 (100.0)	5310 (100.0)	1513 (100.0)

PTB: pulmonary tuberculosis; EPTB: extrapulmonary tuberculosis (**Note:** Totals do not add up due to missing values in each variable).

Table 2. Types of extrapulmonary samples and EPTB positives in three years (2014–16).

Sample types	2014		2015		2016	
	Total	Positive (%)	Total	Positive (%)	Total	Positive (%)
Urine	289	23 (8.0)	195	21 (10.8)	81	1 (1.2)
FNAC	244	22 (9.0)	594	58 (9.8)	796	99 (12.4)
Pleural Fluid	122	6 (4.9)	148	1 (0.7)	215	0 (0.0)
Ascitic Fluid	61	0 (0.0)	83	1 (1.2)	117	0 (0.0)
Peritoneal Fluid	9	0 (0.0)	16	0 (0.0)	0	0 (0.0)
Joint fluid	7	0 (0.0)	7	0 (0.0)	8	0 (0.0)
Pericardial Fluid	2	0 (0.0)	0	0 (0.0)	0	0 (0.0)
Gastric Aspirate	7	0 (0.0)	32	0 (0.0)	80	0 (0.0)
Other aspirates	3	0 (0.0)	0	0 (0.0)	7	1 (14.0)
CSF	9	0 (0.0)	45	0 (0.0)	97	5 (5.2)
Pus	36	7 (19.4)	44	2 (4.5)	65	8 (12.3)
Stool	2	0 (0.0)	5	1 (0.2)	6	0 (0.0)
OT sample	4	0 (0.0)	17	0 (0.0)	41	2 (4.9)
Corneal scraping	1	0 (0.0)	0	0 (0.0)	0	0 (0.0)
Total	796	58 (7.3)	1186	84 (7.1)	1513	116 (7.7)

FNAC: Fine needle aspiration cytology; CSF: Cerebrospinal fluid; OT: Operation theatre.

2016 were 70.1%, 53.8% and 58.5%, respectively. In second follow up at 4 months during the same years were 68.4%, 78.3%, 76.3% respectively (Table 3).

Overall, the total number of sputum samples examined and the total number of patients positive for PTB has increased over the three years although the positivity rates remained consistent. A higher number of patients were tested for PTB during the months of March–April in all the study years (Figure 2).

The sample satisfactory rate varied between 51.4% to 79.0% in the primary samples. A third of the samples in each of the three sputum samples maintained satisfaction rate above 70.0% (Table 4, part A). Sample completeness for PTB of the primary sample was 62.3% in 2014, 94.6% in 2015 and 80.8% in 2016. However, for the subsequent first and second follow up samples, sample completeness dropped sequentially (Table 4, part B). Sample completeness of urine samples for EPTB were 84.8% (245/289), 90.3% (176/195) and 76.5% (62/81) in 2014, 2015 and 2016 respectively.

4. Discussion

This is the first study in Bhutan to determine the TB epidemiological trends, sputum sample adequacy (by quality and number) and conversion rate in PTB patients. By examining the three-year data, the present study has demonstrated increasing investigations of TB suspects, increasing PTB patients and substantial variability in sputum completeness and sample satisfactory rate amongst TB patients investigated in the JDWNRH. Since the JDWNRH is the apex tertiary hospital in Bhutan catering to patients from all over Bhutan, these findings could be a good representation of the country.

This study confirmed the occurrence of TB in younger age groups in the country. In addition, children are affected significantly by both PTB and EPTB. These findings confirm other previous studies on demographic pattern of TB patients and imply that the NTCP should have targeted activities for an effective program. Examination of sputum and non-sputum samples by microscopy remains the main method for diagnosis

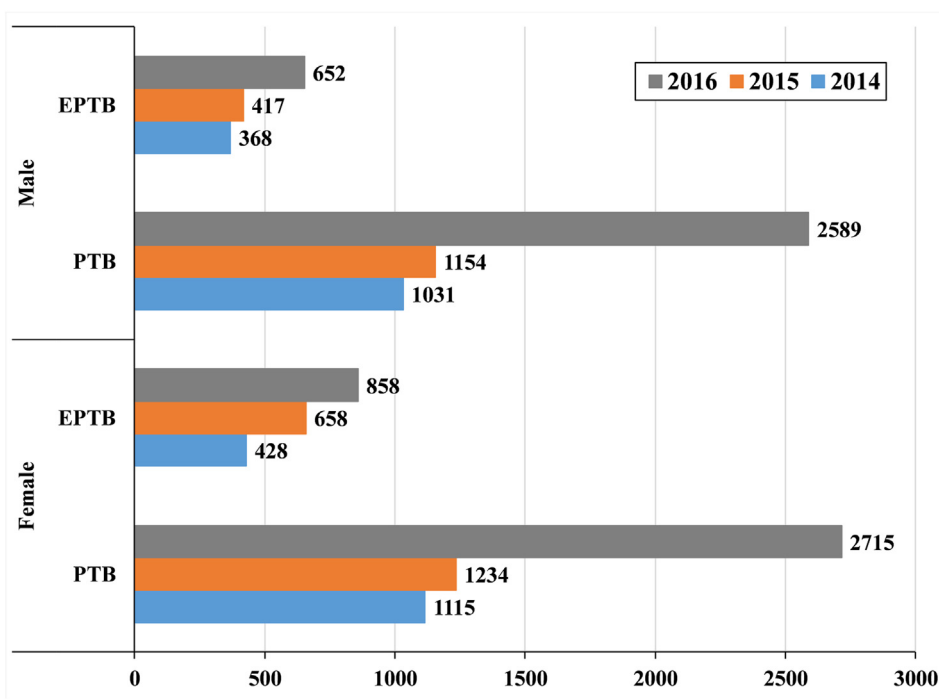


Figure 1. Yearly PTB and EPTB samples examined stratified by gender (PTB: Pulmonary tuberculosis; EPTB: Extra-pulmonary tuberculosis).

Table 3. Sputum conversion rates in follow up samples by year (2014–16).

Sample	2014			2015			2016		
	Follow-up	Negative	Rate (%)	Follow-up	Negative	Rate (%)	Follow-up	Negative	Rate (%)
First follow up*	127	89	70.1	130	70	53.8	289	169	58.5
Second follow up†	38	26	68.4	60	47	78.3	169	129	76.3

* Positive sputum during the primary investigation is the denominator for first (2 months) follow up.

† Positive sputum during the first follow up is the the denominator for second follow up (4 months).

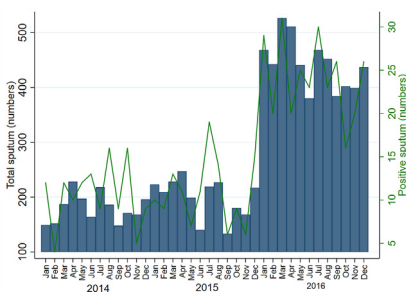


Figure 2. Total number of sputum samples examined and number positive for PTB by year (2014–16).

of TB in the public health system in developing countries including Bhutan. This emphasizes the need to have quality-controlled procedures with competent technical staff to carry out microscopic examination of samples for TB in hospital laboratories. In addition, the patients (and the public) should have adequate awareness on the importance of submitting good quality sputum samples. These would improve the diagnosis of TB by microscopy and contribute to adequate treatment and prevention of the infection. However, the challenges are enormous in resource-constrained countries like Bhutan.

In this study, sample completeness was lowest in 2014 with only about 62.3% of the patients submitting all three samples. This observation demonstrates a lack of knowledge of the patients on the importance

of submitting all three samples for the diagnosis of PTB. To the knowledge of the investigators, currently, there is no internationally accepted standard on the proportion of satisfactory or unsatisfactory sputum samples collected from patients for TB microscopy. In addition, amongst those who submitted sputum samples, there was a high proportion of unacceptable sputum samples. Therefore, there is a need to educate patients on the importance of submitting the required number and good quality of sputum samples. This could be done by providing correct instructions by the health professionals including laboratory technicians. There are a number of consequences associated with inadequate (by number and quality) sputum collections resulting in loss of time and cost associated with slide preparation and examination of poor-quality sputum, failure to diagnosis PTB and increasing the spread of TB in the community.

Urine AFB by ZN stain has questionable sensitivity and specificity due to contamination from environmental saprophytic mycobacteria [13]. It is not widely used as a stand-alone diagnostic test in developed countries. However, in Bhutan, urinary TB diagnosis is heavily depended on microscopic findings of ZN stain of urine for AFB. This practice may have resulted in over-diagnosis and treatment of patients for urinary TB and requires the institution of other more specific methods such as urine lipoarabinomannan (LAM) assays and molecular methods [14]. Urine microscopy for TB should only be used as an adjunct method to other tests. In addition, clinical samples for AFB from sites other than lung and urine also require expert interpretation and adequate clinical correlation.

The sputum conversion rates amongst PTB patients at both first (2 months) and second (4 months) follow-up in this study was lower than

Table 4. Sputum sample adequateness by quality and number of samples (2014–2016).

Year		2014		2015		2016	
A. Sputum sample quality		S (%)	US (%)	S (%)	US (%)	S (%)	US (%)
Primary Sample	Sample 1	1,476 (71.9)	577 (28.1)	1,651 (70.4)	694 (29.6)	3,755 (71.9)	1,465 (28.1)
	Sample 2	1,428 (79.0)	379 (21.0)	1,794 (77.6)	517 (22.4)	3,811 (78.6)	1,036 (21.4)
	Sample 3	1,011 (73.1)	372 (26.9)	1,591 (70.2)	675 (29.8)	2,963 (68.9)	1,337 (31.1)
First follow up	Sample 1	70 (73.7)	25 (26.3)	107 (67.7)	51 (32.3)	201 (69.3)	89 (30.7)
	Sample 2	68 (76.4)	21 (23.6)	119 (75.8)	38 (24.2)	199 (70.6)	83 (29.4)
	Sample 3	34 (73.9)	12 (26.1)	31 (56.4)	24 (43.6)	126 (73.7)	45 (26.3)
Second follow up	Sample 1	31 (60.8)	20 (39.2)	39 (55.7)	31 (44.3)	82 (60.7)	53 (39.3)
	Sample 2	34 (66.7)	17 (33.3)	36 (51.4)	34 (48.6)	74 (57.4)	55 (42.6)
	Sample 3	15 (88.2)	2 (11.8)	16 (61.5)	10 (38.5)	39 (65.0)	21 (35.0)
B. Sample completeness		Complete (%)	Incomplete (%)	Complete (%)	Incomplete (%)	Complete (%)	Incomplete (%)
Primary sample		1,348 (62.3)	816 (37.7)	2,261 (94.6)	129 (5.4)	4,292 (80.8)	1,018 (19.2)
First follow up		39 (30.7)	88 (69.3)	107 (82.3)	23 (17.7)	166 (57.4)	123 (42.6)
Second follow up		15 (11.8)	112 (88.2)	26 (20.0)	104 (80.0)	59 (20.4)	230 (79.6)

S: satisfactory; US: Unsatisfactory.

that observed in Burkina Faso (92.1%) [15], Uganda (76.0%) [16], Tanzania (98.6%) [17], China (95.0%) [18], Cameroon (86.6%) [19] and Taiwan (80.0%) [20] but higher than observed in Morocco (42%) [21]. Non-conversion to sputum negativity with adequate treatment even in first (2 months) and second (4 months) follow-up of sputum positive patients poses great risks to the relatives, healthcare workers and the community. This could result from several factors including non-compliance to medications and drug resistance [22]. However, non-compliance is unlikely since all sputum positive patients are usually admitted to hospitals and treated with DOTS during the intensive phase. This observation will have huge impact on the duration of managing TB patients in the hospital. Increasing MDR-TB has been documented in the country posing an increasing threat to free healthcare in the country [4]. MDR-TB rate in Bhutan is even higher than countries like Rwanda with an estimated prevalence of 3.9% among new smear-positive PTB patients [23]. MDR-TB causing non-conversion of sputum has been associated with inferior prognosis compared to those converting at two months follow-up [24]. However, sputum non-conversion at two months have been proven to have more adverse outcome even in non-MDR-TB patients [25].

An increased number of samples were examined in March and April every year in the current study. This must have resulted from awareness campaigns through multiple sources during the observation of world TB day in the month of March, every year. This finding implicates that such awareness activities are helpful in convincing people to come for disease screening to hospitals.

The NTCP has been actively working towards TB elimination in the country from strengthening case detection, improving treatment compliance and outcomes, public health awareness and instituting diagnostic capacity. The program also carried out surveys that focused mostly on clinical case detection and program effectiveness, with little attention given to assessing the performance of laboratories. The basic requirement for TB diagnosis and control remains with clinical case detection, laboratory confirmation through microscopy or other available facilities and strict institution of DOTs and follow up. Therefore, it is pertinent to strengthen infrastructure and human resource capacity for adequate response to the TB epidemic. Inputs from this study could assist the NTCP understand the educational needs of the patients and public, review the need for the introduction of more sensitive methods like fluorochrome stains and LED microscopy, review the performance of the technical staff in sputum microscopy and institute refresher training as required. In addition, poor sputum conversion in subsequent follow up emphasizes the need of patient isolation during treatment, review compliance and early screening for drug resistance.

This study had few important limitations. First, some sputum samples from OPD were investigated for medical certificates (for different purposes) even though these people did not have signs and symptoms of tuberculosis. Second, a small number may have been included repeatedly, but efforts were taken to identify and exclude them in subsequent examinations. Lastly, in many descriptions and tables, the totals do not add up since entries with missing data were excluded from the statistical calculation for that specific variable.

5. Conclusion

Tuberculosis positivity rate was about 6% for PTB and 7% for EPTB in the JDWNRH during the study period. However, there was significant variation in sputum sample quality and completeness during sample collection which may have impacted the positivity rates. In addition, sputum conversion in first and second follow-up was lower than expected. There is an urgent need to improve TB diagnostics in the country. Public education on the importance of providing adequate samples by number and quality would improve diagnosis of TB, enhance early management, reduce transmission and contribute to elimination of TB.

Declarations

Author contribution statement

T. Tshokey and K. Wangdi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

P. Wangdi, T. Tsheten, S. Pheljay, P. Dema and U. Choden: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors would like to acknowledge the Ministry of Health and the JDWNRH for allowing us to use the TB data for this study. We also thank all the patients whose data were utilized for this study.

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